

- 314 -

CLAIMS

What is Claimed is:

- 5 1. An isolated serine protease obtained from a member of the *Micrococcineae*.
2. The serine protease of Claim 1, wherein said protease is a cellulomonadin.
3. The serine protease of Claim 1, wherein said protease is obtained from an
10 organism selected from the group consisting of *Cellulomonas*, *Oerskovia*,
Cellulosimicrobium, *Xylanibacterium*, and *Promicromonospora*.
4. The serine protease of Claim 3, wherein said protease is obtained from
Cellulomonas 69B4.
- 15 5. The serine protease of Claim 4, wherein said protease comprises the amino
acid sequence set forth in SEQ ID NO:8.
6. A composition comprising an isolated serine protease having immunological
20 cross-reactivity with said serine protease of Claim 4.
7. A composition comprising an isolated serine protease having immunological
cross-reactivity with said serine protease of Claim 1.
- 25 8. An isolated serine protease comprising at least 60% amino acid identity with
said serine protease of Claim 5.
9. The amino acid sequence of Claim 4, wherein said sequence comprises
substitutions at least one amino acid position selected from the group comprising positions
30 2, 8, 10, 11, 12, 13, 14, 15, 16, 24, 26, 31, 33, 35, 36, 38, 39, 40, 43, 46, 49, 51, 54, 61, 64,
65, 67, 70, 71, 76, 78, 79, 81, 83, 85, 86, 90, 93, 99, 100, 105, 107, 109, 112, 113, 116,
118, 119, 121, 123, 127, 145, 155, 159, 160, 163, 165, 170, 174, 179, 183, 184, 185, 186,
187, and 188.
- 35 10. The amino acid sequence of Claim 4, wherein said sequence comprises

- 315 -

substitutions at least one amino acid position selected from the group comprising positions 1, 4, 22, 27, 28, 30, 32, 41, 47, 48, 55, 59, 63, 66, 69, 75, 77, 80, 84, 87, 88, 89, 92, 96, 110, 111, 114, 115, 117, 128, 134, 144, 143, 146, 151, 154, 156, 158, 161, 166, 176, 177, 181, 182, 187, and 189.

5

11. An isolated protease variant having an amino acid sequence comprising at least one substitution of an amino acid made at a position equivalent to a position in a *Cellulomonas* 69B4 protease comprising the amino acid sequence set forth in SEQ ID NO:8.

10

12. The isolated protease of Claim 11, wherein said substitutions are made at positions equivalent to positions 2, 8, 10, 11, 12, 13, 14, 15, 16, 24, 26, 31, 33, 35, 36, 38, 39, 40, 43, 46, 49, 51, 54, 61, 64, 65, 67, 70, 71, 76, 78, 79, 81, 83, 85, 86, 90, 93, 99, 100, 105, 107, 109, 112, 113, 116, 118, 119, 121, 123, 127, 145, 155, 159, 160, 163, 165, 170, 174, 179, 183, 184, 185, 186, 187, and 188 in a *Cellulomonas* 69B4 protease comprising an amino acid sequence set forth in SEQ ID NO:8.

15

13. The isolated protease of Claim 11, wherein said substitutions are made at positions equivalent to positions 1, 4, 22, 27, 28, 30, 32, 41, 47, 48, 55, 59, 63, 66, 69, 75, 77, 80, 84, 87, 88, 89, 92, 96, 110, 111, 114, 115, 117, 128, 134, 144, 143, 146, 151, 154, 156, 158, 161, 166, 176, 177, 181, 182, 187, and 189, in a *Cellulomonas* 69B4 protease comprising an amino acid sequence set forth in SEQ ID NO:8.

20

14. An isolated protease comprising the amino acid sequence set forth in SEQ ID NO:8, wherein at least one amino acid position at positions selected from the group consisting of 14, 16, 35, 36, 65, 75, 76, 79, 123, 127, 159, and 179, are substituted with another amino acid.

25

15. The protease of Claim 14, wherein said protease comprises at least one mutation selected from the group consisting of R14L, R16I, R16L, R16Q, R35F, T36S, G65Q, Y75G, N76L, N76V, R79T, R123L, R123Q, R127A, R127K, R127Q, R159K, R159Q, and R179Q.

30

16. The protease of Claim 15, wherein said protease comprises multiple mutations selected from the group consisting of R16Q/R35F/R159Q, R16Q/R123L, R14L/R127Q/R159Q, R14L/R179Q, R123L/R127Q/R179Q, R16Q/R79T/R127Q, and

35

- 316 -

R16Q/R79T.

17. The protease of Claim 15, wherein said protease comprises the following mutations R123L, R127Q, and R179Q.

18. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of T36I, A38R, N170Y, N73T, G77T, N24A, T36G, N24E, L69S, T36N, T36S, E119R, N74G, T36W, S76W, N24T, N24Q, T36P, S76Y, T36H, G54D, G78A, S187P, R179V, N24V, V90P, T36D, L69H, G65P, G65R, N7L, W103M, N55F, G186E, A70H, S76V, G186V, R159F, T36Y, T36V, G65V, N24M, S51A, G65Y, Q71I, V66H, P118A, T116F, A38F, N24H, V66D, S76L, G177M, G186I, H85Q, Q71K, Q71G, G65S, A38D, P118F, A38S, G65T, N67G, T36R, P118R, S114G, Y75I, I181H, G65Q, Y75G, T36F, A38H, R179M, T183I, G78S, A64W, Y75F, G77S, N24L, W103I, V3L, Q81V, R179D, G54R, T36L, Q71M, A70S, G49F, G54L, G54H, G78H, R179I, Q81K, V90I, A38L, N67L, T109I, R179N, V66I, G78T, R179Y, S187T, N67K, N73S, E119K, V3I, Q71H, I11Q, A64H, R14E, R179T, L69V, V150L, Q71A, G65L, Q71N, V90S, A64N, I11A, N145I, H85T, A64Y, N145Q, V66L, S92G, S188M, G78D, N67A, N7S, V80H, G54K, A70D, P118H, D2G, G54M, Q81H, D2Q, V66E, R79P, A38N, N145E, R179L, T109H, R179K, V66A, G54A, G78N, T109A, R179A, N7A, R179E, H104K, A64R, and V80L.

19. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of H85R, H85L, T62I, N67H, G54I, N24F, T40V, T86A, G63V, G54Q, A64F, G77Y, R35F, T129S, R61M, I126L, S76N, T182V, R79G, T109P, R127F, R123E, P118I, T109R, I71S, T183K, N67T, P89N, F1T, A64K, G78I, T109L, G78V, A64M, A64S, T10G, G77N, A64L, N67D, S76T, N42H, D184F, D184R, S76I, S78R, A38K, V72I, V3T, T107S, A38V, F47I, N55Q, S76E, P118Q, T109G, Q71D, P118K, N67S, Q167N, N145G, I28L, I11T, A64I, G49K, G49A, G65A, N170D, H85K, S185I, I181N, V80F, L69W, S76R, D184H, V150M, T183M, N67Q, S51Q, A38Y, T107V, N145T, Q71F, A83N, S76A, N67R, T151L, T163L, S51F, Q81I, F47M, A41N, P118E, N67Y, T107M, N73H, 67V, G63W, T10K, I181G, S187E, T107H, D2A, L142V, A143N, A8G, S187L, V90A, G49L, N170L, G65H, T36C, G12W, S76Q, A143S, F1A, N7H, S185V, A110T, N55K, N67F, N7I, A110S, N170A, Q81D, A64Q, Q71L, A38I, N112I, V90T, N145L, A64T, I11S, A30S, R123I, D2H, V66M, Q71R, V90L, L68W, N24S, R159E, V66N, D184Q, E133Q, A64V, D2N, G13M, T40S, S76K, G177S, G63Q, S15F, A8K, A70G, and

- 317 -

A38G.

20. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of R35E, R35D, R14E, R14D, Q167E, G49C, S15R, S15H, I11W, S15C, G49Q, R35Q, R35V, G49E, R123D, R123Y, G49H, A38D, R35S, F47R, R123C, T151L, R14T, R35T, R123E, G49A, G49V, D56L, R35N, R35A, G12D, R35C, R123N, T46V, R123H, S155C, T121E, R127E, S113C, R123T, R16E, T46F, T121L, A38C, T46E, R123W, T44E, N55G, A8G, E119G, R35P, R14G, F59W, R127S, R61E, R14S, S155W, R123F, R123S, G49N, R127D, E119Y, A48E, N170D, R159T, S99A, G12Q, P118R, F165W, R127Q, R35H, G12N, A22C, G12V, R16T, Y57G, T100A, T46Y, R159E, E119R, T107R, T151C, G54C, E119T, R61V, I11E, R14I, R61M, S15E, A22S, R16C, T36C, R16V, L125Q, M180L, R123Q, R14A, R14Q, R35M, R127K, R159Q, N112P, G124D, R179E, G49L, A41D, G177D, R123V, E119V, T10L, T109E, R179D, G12S, T10C, G91Q, S15Y, S155Y, R14C, T163D, T121F, R14N, F165E, N24E, A41C, R61T, G12I, P118K, T46C, I11T, R159D, N170C, R159V, S155I, I11Q, D2P, T100R, R159S, S114C, R16D, and P134R.

21. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of S99G, T100K, R127A, F1P, S155V, T128A, F165H, G177E, A70M, S140P, A87E, D2I, R159K, T36V, R179C, E119N, T10Y, I172A, A8T, F47V, W103L, R61K, D2V, R179V, D2T, R159N, E119A, G54E, R16Q, G49S, R16I, S51L, S155E, S15M, R179I, T10Q, G12H, R159C, R179T, T163C, R159A, A132S, N157D, G13E, L141M, A41T, R123M, R14M, A8R, Q81P, N24T, T10D, A88F, R61Q, S99K, R179Y, T121A, N112E, S155T, T151V, S99Q, T10E, S92T, T109K, T44C, R123A, A87C, S15F, S155F, D56F, T10F, A83H, R179M, T121D, G13D, P118C, G49F, Q174C, S114E, T86E, F1N, T115C, R127C, R123K, V66N, G12Y, S113A, S15N, A175T, R79T, R123G, R179S, R179N, R123I, P118A, S187E, N112D, A70G, E119L, E119S, R159M, R14H, R179F, A64C, A41S, R179W, N24G, T100Q, P118W, Q81G, G49K, R14L, N55A, R35K, R79V, D2M, T160D, A83D, R179L, S51A, G12P, S99H, N42D, S188E, T10M, L125M, T116N, A70P, Q174S, G65D, S113D, E119Q, A83E, N170L, Q81A, S51C, P118G, Q174T, I28V, S15G, and T116G.

22. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of G26I, G26K, G26Q, G26V, G26W, F27V, F27W, I28P, T29E, T129W, T40D, T40Q, R43D, P43H, P43K,

- 318 -

P43L, A22C, T40H, P89W, G91L, S18E, F59K, A30M, A30N, G31M, C33M, G161L, G161V, P43N, G26E, N73P, G84C, G84P, G45V, C33L, Y9E, Y9P, A147E, C158H, I28W, A48P, A22S, T62R, S137R, S155P, S155R, G156I, G156L, Q81A, R96C, I4D, I4P, A70P, C105E, C105G, C105K, C105M, C105N, C105S, T128A, T128V, T128G, S140P, G12D, C33N, C33E, T164G, G45A, G156P, S99A, Q167L, S155W, I28T, R96F, A30P, R123W, T40P, T39R, C105P, T100A, C105W, S155K, T46Y, R123F, I4G, S155Y, T46V, A93S, Y57N, Q81S, G186S, G31H, T10Y, G31V, A83H, A38D, R123Y, R79T, C158G, G31Y, Q81P, R96E, A30Y, R159K, A22T, T40N, Y57M, G31N, Q81G, T164L, T121E, T10F, Q146P, R123N, V3R, P43G, Q81H, Q81D, G161I, C158M, N24T, T10W, T128S, T160I, Y176P, S155F, T128C, L125A, P168Y, T62G, F166S, S188A, Q81F, T46W, A70G, and A38G.

23. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of S188E, S188V, Y117K, Y117Q, Y117R, Y117V, R127K, R127Q, R123L, T86S, R123I, Q81E, L125M, H32A, S188T, N74F, C33D, F27I, A83M, Q71Y, R123T, V90A, F59W, L141C, N170E, T46F, S51V, G162P, S185R, A41S, R79V, T151C, T107S, T129Y, M180L, F166C, C105T, T160E, P89A, R159T, T183P, S188M, T10L, G25S, N24S, E119L, T107L, T107Q, G161K, G15Q, S15R, G153K, G153V, S188G, A83E, G186P, T121D, G49A, S15C, C105Y, C105A, R127F, Q71A, T10C, R179K, T86I, W103N, A87S, F166A, A83F, R123Q, A132C, A143H, T163I, T39V, A93D, V90M, R123K, P134W, G177N, V115I, S155T, T110D, G105L, N170D, T107A, G84V, G84M, L111K, P168I, G154L, T183I, S99G, S15T, A8G, S15N, P189S, S188C, T100Q, A110G, A121A, G12A, R159V, G31A, G154R, T182L, V115L, T160Q, T107F, R159Q, G144A, S92T, T101S, A83R, G12HM S15H, T116Q, T36V, G154, Q81C, V130T, T183A, P118T, A87E, T86M, V150N, and N24E.

24. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of T36I, I172T, N24E, N170Y, G77T, G186N, I181L, N73T, A38R, N74G, N24A, G54D, S76D, R123E, I59E, N112E, R35E, R179V, R123D, N24T, R179T, R14L, A38D, V90P, R14Q, R123I, R179D, S76V, R79G, R35L, S76E, S76Y, R79D, R79P, R35Q, R179N, N112D, R179E, G65P, Y75G, V90S, R179M, R35F, R123F, A64I, N24Q, R14I, R179A, R127A, R179I, N170D, R35A, R159F, T109E, R14D, N67D, G49A, N112Q, G78D, T121E, L69S, T116E, V90I, T36S, T36G, N145E, T86D, S51D, R179K, T107E, T129S, L142V, R79A, R79E, A38H, T107S, R123A, N55E, R123L, R159N, G65D, R14N, G65Q, R123Q, N24V, R14G, T116Q, A38N, R159Q, R179Y, A83E, N112L, S99N, G78A, T10N, H85Q, R35Q, N24L, N24H,

- 319 -

G49S, R79L, S76T, S76L, G65S, N55F, R79V, G65T, R123N, T86E, Y75F, F1T, S76N, S99V, R79T, N112V, R79M, T107V, R79S, G54E, G65V, R127Q, R159D, T107H, H85T, R35T, T36N, Q81E, R123H, S76I, A38F, V90T, and R14T.

5 25. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of G65L, S99D, T107M, S113T, S99T, G77S, R14M, A64N, R61M, A70D, Q71G, A93D, S92G, N112Y, S15W, R159K, N67G, T10E, R127H, A64Y, R159C, A38L, T160E, T183E, R127S, A8E, S51Q, N7L, G63D, A38S, R35H, R14K, T107I, G12D, A64L, S76W, A41N, R35M, A64V,
10 A38Y, T183I, W103M, A41D, R127K, T36D, R61T, G65Y, G13S, R35Y, R123T, A64H, G49H, A70H, A64F, R127Y, R61E, A64P, T121D, V115A, R123Y, T101S, T182V, H85L, N24M, R127E, N145D, Q71H, S76Q, A64T, G49F, A64Q, T10D, F1D, A70G, R35W, Q71D, N121I, A64M, T36H, A8G, T107N, R35S, N67T, S92A, N170L, N67E, S114A, R14A, R14S, Q81D, S51H, R123S, A93S, R127F, I19V, T40V, S185N, R123G, R179L, S51V, T163D,
15 T109I, A64S, V72I, N67S, R159S, H85M, T109G, Q71S, R61H, T107A, Q81V, V90N, T109A, A38T, N145T, R159A, A110S, Q81H, A48E, S51T, A64W, R159L, N67H, A93E, T116F, R61S, R123V, V3L, and R159Y.

26. The protease of Claim 11, wherein the amino acid sequence of said protease
20 comprises at least one substitution selected from the group consisting of T36I, P89D, A93T, A93S, T36N, N73T, T36G, R159F, T36S, A38R, S99W, S76W, T36P, G77T, G54D, R127A, R159E, H85Q, T36D, S76L, S99N, Y75G, S76Y, R127S, N24E, R127Q, D184F, N170Y, N24A, S76T, H85L, Y75F, S76V, L69S, R159K, R127K, G65P, N74G, R159H, G65Q, G186V, A48Q, T36H, N67L, R14I, R127L, T36Y, S76I, S114G, R127H, S187P, V3L, G78D,
25 R123I, I181Q, R35F, H85R, R127Y, N67S, Q81P, R123F, R159N, S99A, S76D, A132V, R127F, A143N, S92A, N24T, R79P, S76N, R14M, G186E, N24Q, N67A, R127T, H85K, G65T, G65Y, R179V, Y75I, I11Q, A38L, T36L, R159Y, R159D, N24V, G65S, N157D, G186I, G54Q, N67Y, R127G, S76A, A38S, T109E, V66H, T116F, R123L, G49A, A64H, T36W, D184H, S99D, G161K, P134E, A64F, N67G, S99T, D2Q, S76E, R16Q, G54N,
30 N67V, R35L, Q71I, N7L, N112E, L69H, N24H, G54I, R16L, N24M, A64Y, S113A, H85F, R79G, I11A, T121D, R61V, and G65L.

27. The protease of Claim 11, wherein the amino acid sequence of said protease
comprises at least one substitution selected from the group consisting of N67Q, S187Q,
35 Q71H, T163D, R61K, R159V, Q71F, V31F, V90I, R79D, T160E, R123Q, A38Y, S113G,

- 320 -

A88F, A70G, I11T, G78A, N24L, S92G, R14L, D184R, G54L, N112L, H85Y, R16N, G77S, R179T, V80L, G65V, T121E, Q71D, R16G, P89N, N42H, G49F, I11S, R61M, R159C, G65R, T183I, A93D, L111E, S51Q, G78N, N67T, A38N, T40V, A64W, R159L, T10E, R179K, R123E, V90P, A64N, G161E, H85T, A8G, L142V, A41N, S185I, Q71L, A64T, R16I,
5 A38D, G54M, N112Q, R16A, R14E, V80H, N170D, S99G, R179N, S15E, G49H, A70P, A64S, G54A, S185W, R61H, T10Q, A38F, N170L, T10L, N67F, G12D, D184T, R14N, S187E, R14P, N112D, S140A, N112G, G49S, L111D, N67M, V150L, G12Y, R123K, P89V, V66D, G77N, S51T, A8D, I181H, T86N, R179D, N55F, N24S, D184L, R61S, N67K, G186L, F1T, R159A, I11L, R61T, D184Q, A93E, Q71T, R179E, L69W, T163I, S188Q, L125V,
10 A38V, R35A, P134G, A64V, N145D, V90T, and A143S.

28. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of T36I, N170Y, A38R, R79P, G77T, L69S, N73T, S76V, S76Y, R179V, T36N, N55F, R159F, G54D, G65P,
15 L69H, T36G, G177M, N24E, N74G, R159E, T36S, Y75G, S76I, S76D, A8R, A24A, V90P, R159C, G65Q, T121E, A8V, S76L, T109E, R179M, A8T, T107N, G186E, S76W, R123E, A38F, T36P, N67G, Y75F, S76N, R179I, S187P, N67V, V90S, R127A, R179Y, R35F, N145S, G65S, R61M, S51A, R179N, R123D, N24T, N55E, R79C, G186V, R123I, G161E, G65Y, A38S, R14L, V90I, R79G, N145E, N67L, R127S, R150Y, M180D, N67T, A93D,
20 T121D, Q81V, T109I, A93E; T107S, R179T, R179L, R179K, R159D, R179A, R79E, R123F, R79D, T36D, A64N, L142V, T109A, I172V, A83N, T85A, R179D, A38L, I126L, R127Q, R127L, L69W, R127K, G65T, R127H, P134A, N67D, R14M, N24Q, A143N, N55S, N67M., S51D, S76E, T163D, A38D, R159K, T183I, G63V, A8S, T107M, H85Q, N112E, N67F, N67S, A64H, T86I, P134E, T182V, N67Y, A64S, G78D, V90T, R61T, R16Q, G65R, T86L,
25 V90N, R159Q, G54I, S76C, R179E, V66D, L69V, R127Y, R35L, R14E, and T86F.

29. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of G186I, A64Q, T109G, G64L, N24L, A8E, N112D, A38H, R179W, S114G, R123L, A8L, T129S, N170D,
30 R159N, N67C, S92C, T107A, G54E, T107E, T36V, R127T, A8N, H85L, A110S, N170C, A64R, A132V, T36Y, G63D, W103M, T151V, R123P, W103Y, S76T, S187T, R127F, N67A, P171M, A70S, R159H, S76Q, L125V, G54Q, G49L, R14I, R14Q, A83I, V90L, T183E, R159A, T101S, G65D, G54A, T107Q, Q71M, T86E, N24M, N55Q, R61V, P134D, R96K, A88F, N145Q, A64M, A64T, N24V, S140A, A8H, A64I, R123Q, T183Q, N24H, A64W, T62I,
35 T129G, R35A, T40V, I11T, A38N, N145G, A175T, G77Q, T109H, A8P, R35E, T109N,

- 321 -

A110T, N67Q, G63P, H85R, S92G, A175V, S51Q, G63Q, T116F, G65A, R79L, N145P, L69Q, Q146D, A83D, F166Y, R123A, T121L, R123H, A70P, T182W, S76A, A64F, T107H, G186L, Q81I, R123K, A64L, N67R, V3L, S187E, S161K, T86M, I4M, G77N, G49A, A41N, G54M, T107V, Q81E, A38I, T109L, T183K, A70G, Q71D, T183L, Q81H, A64V, A93Q, S188E, S51F, G186P, G186T, R159L, P134G, N145T, N55V, V66E, R159V, Y176L, and R16L.

30. The protease of Claim 11, wherein the amino acid sequence of said protease comprises at least one substitution selected from the group consisting of T36I, N73T, P134R, G77T, N24E, P134E, P134L, N24T, 159F, L69S, T10G, G186S, S140A, T36S, N112S, N24Q, T36G, P134H, G34A, N24A, A38T, E119R, G186E, R14M, S76W, T10A, A38F, L142V, N170Y, P134V, A22V, S76V, T182V, S76Y, I11A, I11S, S118A, G186V, L69H, I11T, T36N, G65V, G49F, V90I, R179V, R16K, T163I, R127F, R159K, N24L, Q71I, S15G, S15F, R14G, S99N, T10L, S15E, T107R, F166Y, G49A, V90P, P134D, Q167N, S76D, S51A, V80A, V150L, N74G, T107K, S76L, N24V, G12I, S99V, and R16N.

31. The protease of Claim 11, wherein the amino acid of said protease comprises Arg14, Ser15, Arg16, Cys17, His32, Cys33, Phe52, Asp56, Thr100, Val115, Thr116, Tyr117, Pro118, Glu119, Ala132, Glu133, Pro134, Gly135, Asp136, Ser137, Thr151, Ser152, Gly153, Gly154, Ser155, Gly156, Asn157, Thr164, and Phe165.

32. The protease of Claim 31, wherein the catalytic triad of said protease comprises His 32, Asp56, and Ser137.

33. The protease of Claim 11, wherein the amino acid sequence of said protease comprises Cys131, Ala132, Glu133, Pro134, Gly135, Thr151, Ser152, Gly153, Gly154, Ser155, Gly156, Asn157 and Gly 162, Thr 163, and Thr164.

34. The protease of Claim 11, wherein the amino acid sequence of said protease comprises Phe52, Tyr117, Pro118 and Glu119.

35. The protease of Claim 11, wherein the amino acid sequence of said protease has main-chain to main-chain hydrogen bonding from Gly 154 to the substrate main-chain.

36. The protease of Claim 11, wherein said protease comprises three disulfide

- 322 -

bonds.

37. The protease of Claim 11, wherein said variant has an altered substrate specificity as compared to wild-type *Cellulomonas* 69B4 protease.

5

38. The protease of Claim 11, wherein said variant has an altered pI as compared to wild-type *Cellulomonas* 69B4 protease.

39. The protease of Claim 11, wherein said variant has improved stability as compared to wild-type *Cellulomonas* 69B4 protease.

10

40. The protease of Claim 11, wherein said variant exhibits an altered surface property.

41. The protease of Claim 40, wherein said variant comprises mutations at least one substitution at sites selected from the group consisting of 1, 2, 4, 7, 8, 10, 11, 12, 13, 14, 15, 16, 22, 24, 25, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 57, 59, 61, 62, 63, 64, 65, 66, 67, 68, 69, 71, 73, 74, 75, 76, 77, 78, 79, 80, 81, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 95, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 123, 124, 126, 127, 128, 130, 131, 132, 133, 134, 135, 137, 143, 144, 145, 146, 147, 148, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 170, 171, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, and 184.

15

20

42. The protease of Claim 1, wherein said protease is a variant protease having at least one improved property as compared to wild-type protease.

25

43. The protease of Claim 42, wherein said at least one improved property is selected from the group consisting of acid stability, thermostability, casein hydrolysis, keratin hydrolysis, cleaning performance, and LAS stability.

30

44. An expression vector comprising a polynucleotide sequence encoding the protease variant of Claim 11.

45. A host cell comprising said expression vector of Claim 44.

35

- 323 -

46. The host cell of Claim 45, wherein said host is selected from the group consisting of *Bacillus* sp., *Streptomyces* sp., *Aspergillus* sp., and *Trichoderma* sp.

5 47. A serine protease produced by said host cell of Claim 46.

48. A variant protease comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78,

10 49. The variant protease of Claim 42, wherein said amino acid sequence is encoded by a polynucleotide sequence selected from the group consisting of SEQ ID NOS:53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, and 77.

15 50. An expression vector comprising a polynucleotide sequence encoding the protease variant of Claim 49.

51. A host cell comprising said expression vector of Claim 50.

20 52. The host cell of Claim 51, wherein said host is selected from the group consisting of *Bacillus* sp., *Streptomyces* sp., *Aspergillus* sp., and *Trichoderma* sp.

53. A serine protease produced by said host cell of Claim 52.

25 54. A composition comprising at least a portion of the isolated serine protease of Claim 1, wherein said protease is encoded by a polynucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4.

55. The polynucleotide sequence of Claim 54, wherein said sequence comprises at least a portion of SEQ ID NO:1.

30 56. An expression vector comprising the polynucleotide sequence of Claim 55.

57. A host cell comprising said expression vector of Claim 56.

35 58. The host cell of Claim 57, wherein said host is selected from the group

- 324 -

consisting of *Bacillus* sp., *Streptomyces* sp., *Aspergillus* sp., and *Trichoderma* sp.

59. A serine protease produced by said host cell of Claim 58.

5 60. A variant serine protease, wherein said protease comprises at least one substitution corresponding to the amino acid positions in SEQ ID NO:8, and wherein said variant protease has better performance in at least one property selected from the group consisting of keratin hydrolysis, thermostability, casein activity, LAS stability, and cleaning, as compared to wild-type *Cellulomonas* 69B4 protease.

10 61. An isolated polynucleotide comprising a nucleotide sequence (i) having at least 70% identity to SEQ ID NO:4, or (ii) being capable of hybridizing to a probe derived from the nucleotide sequence set forth in SEQ ID NO:4, under conditions of intermediate to high stringency, or (iii) being complementary to the nucleotide sequence set forth in SEQ ID
15 NO:4.

62. A vector comprising the polynucleotide of Claim 61.

63. A host cell transformed with the vector of Claim 62.

20 64. A polynucleotide comprising a sequence complementary to at least a portion of the sequence set forth in SEQ ID NO:4.

65. A method of producing an enzyme having protease activity, comprising:

- 25 (a) transforming a host cell with an expression vector comprising a polynucleotide having at least 70% sequence identity to SEQ ID NO:4;
- (b) cultivating said transformed host cell under conditions suitable for said host cell to produce said protease; and
- (c) recovering said protease.

30 66. The method of Claim 65, wherein said host cell is a *Streptomyces*, *Aspergillus*, *Trichoderma* or *Bacillus* species.

67. A probe comprising a 4 to 150 polynucleotide sequence substantially identical
35 to a corresponding fragment of SEQ ID NO:4, wherein said probe is used to detect a nucleic

- 325 -

acid sequence coding for an enzyme having proteolytic activity, and wherein said nucleic acid sequence is obtained from a member of the *Micrococcineae*.

68. The probe of Claim 67, wherein said *Micrococcineae* is a *Cellulomonas* spp..

69. The probe of Claim 68, wherein said *Cellulomonas* is *Cellulomonas* strain 69B4.

70. A cleaning composition comprising at least one serine protease obtained from a member of the *Micrococcineae*.

71. The cleaning composition of Claim 70, wherein said protease is a serine protease obtained from an organism selected from the group consisting of *Cellulomonas*, *Oerskovia*, *Cellulosimicrobium*, *Xylanibacterium*, and *Promicromonospora*.

72. The cleaning composition of Claim 71, wherein said protease is obtained from *Cellulomonas* 69B4.

73. The cleaning composition of Claim 72, wherein said protease comprises the amino acid sequence set forth in SEQ ID NO:8.

74. The cleaning composition of Claim 73, wherein said serine protease has at least 60% amino acid identity with the amino acid sequence set forth in SEQ ID NO:8.

75. A cleaning composition comprising an serine protease, wherein said serine protease has immunological cross-reactivity with the serine protease of Claim 70.

76. A cleaning composition comprising an serine protease, wherein said serine protease has immunological cross-reactivity with the serine protease of Claim 72.

77. The cleaning composition of Claim 70, wherein said protease is a variant protease having an amino acid sequence comprising at least one substitution of an amino acid made at a position equivalent to a position in a *Cellulomonas* 69B4 protease having an amino acid sequence set forth in SEQ ID NO:8.

- 326 -

78. The cleaning composition of Claim 77, wherein said substitutions are made at positions equivalent to positions 2, 8, 10, 11, 12, 13, 14, 15, 16, 24, 26, 31, 33, 35, 36, 38, 39, 40, 43, 46, 49, 51, 54, 61, 64, 65, 67, 70, 71, 76, 78, 79, 81, 83, 85, 86, 90, 93, 99, 100, 105, 107, 109, 112, 113, 116, 118, 119, 121, 123, 127, 145, 155, 159, 160, 163, 165, 170, 174, 179, 183, 184, 185, 186, 187, and 188 in a *Cellulomonas* 69B4 protease comprising the amino acid sequence set forth in SEQ ID NO:8.

79. The cleaning composition of Claim 77, wherein said substitutions are made at positions equivalent to positions 1, 4, 22, 27, 28, 30, 32, 41, 47, 48, 55, 59, 63, 66, 69, 75, 77, 80, 84, 87, 88, 89, 92, 96, 110, 111, 114, 115, 117, 128, 134, 144, 143, 146, 151, 154, 156, 158, 161, 166, 176, 177, 181, 182, 187, and 189, in a *Cellulomonas* 69B4 protease comprising the amino acid sequence set forth in SEQ ID NO:8.

80. The cleaning composition of Claim 77, wherein said protease comprises at least one amino acid substitutions at positions 14, 16, 35, 36, 65, 75, 76, 79, 123, 127, 159, and 179, in an equivalent amino acid sequence to that set forth in SEQ ID NO:8.

81. The cleaning composition of Claim 80, wherein said protease comprises at least one mutation selected from the group consisting of R14L, R16I, R16L, R16Q, R35F, T36S, G65Q, Y75G, N76L, N76V, R79T, R123L, R123Q, R127A, R127K, R127Q, R159K, R159Q, and R179Q.

82. The cleaning composition of Claim 81, wherein said protease comprises a set of mutations selected from the group consisting of the sets R16Q/R35F/R159Q, R16Q/R123L, R14L/R127Q/R159Q, R14L/R179Q, R123L/R127Q/R179Q, R16Q/R79T/R127Q, and R16Q/R79T.

83. The cleaning composition of Claim 81, wherein said protease comprises the following mutations R123L, R127Q, and R179Q.

84. The cleaning composition of Claim 80, wherein said variant serine protease comprises at least one substitution corresponding to the amino acid positions in SEQ ID NO:8, and wherein said variant protease has better performance in at least one property selected from the group consisting of keratin hydrolysis, thermostability, casein activity, LAS stability, and cleaning, as compared to wild-type *Cellulomonas* 69B4 protease.

- 327 -

85. The cleaning composition of Claim 70, wherein said variant protease comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78,

5

86. The cleaning composition of Claim 70, wherein said variant protease amino acid sequence is encoded by a polynucleotide sequence selected from the group consisting of SEQ ID NOS:53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, and 77.

10

87. A cleaning composition comprising a cleaning effective amount of a proteolytic enzyme, said enzyme comprising an amino acid sequence having at least 70 % sequence identity to SEQ ID NO:4, and a suitable cleaning formulation.

15

88. The cleaning composition of Claim 87, further comprising one or more additional enzymes or enzyme derivatives selected from the group consisting of proteases, amylases, lipases, mannanases, pectinases, cutinases, oxidoreductases, hemicellulases, and cellulases.

20

89. A composition comprising the serine protease of Claim 1 and at least one stabilizing agent.

90. The composition of Claim 89, wherein said stabilizing agent is selected from the group consisting of borax, glycerol, and competitive inhibitors.

25

91. The composition of Claim 90, wherein said competitive inhibitors stabilize said serine protease to anionic surfactants.

92. The composition of Claim 1, wherein said serine protease is an autolytically stable variant.

30

93. A cleaning composition comprising at least 0.0001 weight percent of the serine protease of Claim 1, and optionally, an adjunct ingredient.

35

94. The cleaning composition of Claim 93, wherein said composition comprises an adjunct ingredient.

- 328 -

95. A cleaning composition according to Claim 93, said composition comprising a sufficient amount of a pH modifier to provide said composition with a neat pH of from about 3 to about 5, said composition being essentially free of materials that hydrolyze at a pH of
5 from about 3 to about 5.

96. A cleaning composition according to Claim 95, wherein said materials that hydrolyze comprise a surfactant material.

10 97. A cleaning composition according to Claim 95, said cleaning composition being a liquid composition.

98. A cleaning composition according to Claim 96, wherein said surfactant material comprises a sodium alkyl sulfate surfactant that comprises an ethylene oxide
15 moiety.

99. A cleaning composition that comprises at least one acid stable enzyme, said cleaning composition comprising a sufficient amount of a pH modifier to provide said composition with a neat pH of from about 3 to about 5, said composition being essentially
20 free of materials that hydrolyze at a pH of from about 3 to about 5.

100. A cleaning composition according to Claim 99, wherein said materials that hydrolyze comprise a surfactant material.

25 101. A cleaning composition according to Claim 99, said cleaning composition being a liquid composition.

102. A cleaning composition according to Claim 99, wherein said surfactant material comprises a sodium alkyl sulfate surfactant that comprises an ethylene oxide
30 moiety.

103. A cleaning composition according to Claim 95, said composition comprising a suitable adjunct ingredient.

35 104. The cleaning composition according to Claim 99, said composition

- 329 -

comprising a suitable adjunct ingredient.

105. A composition according to Claim 95, said composition comprising from about 0.001 to about 0.5 weight % of ASP.

5

106. A composition according to Claim 105, said composition from about 0.01 to about 0.1 weight percent of ASP.

107. A method of cleaning, said method comprising the steps of:

10

- a) contacting a surface and/or an article comprising a fabric with the cleaning composition of Claim 94 and/or a composition comprising the cleaning composition of Claim 98; and
- b) optionally washing and/or rinsing said surface or material.

15

108. A method of cleaning, said method comprising the steps of:

- a) contacting a surface and/or an article comprising a fabric with the cleaning composition of Claim 99, and/or a composition comprising the cleaning composition of Claim 100; and
- b) optionally washing and/or rinsing said surface or material.

20

109. An animal feed comprising the serine protease of Claim 1.